Potential Bile Acid Metabolites. XV. Synthesis of 4β-Hydroxylated Bile Acids; Unique Bile Acids in Human Fetal Bile[1)]

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The 4β-hydroxylated derivatives of lithocholic, deoxycholic, chenodeoxycholic, and cholic acids were synthesized from their respective parent compounds. The principal reactions employed were 1) β-face *cis*-dihydroxylation of Δ^3 intermediates with osmium tetroxide-*N*-methylmorpholine *N*-oxide, 2) selective cathylation of vicinal 3β,4β-diols followed by oxidation of the resulting 4β-monocathylates, or direct selective oxidation at C-3 of 3β,4β-diols with pyridinium chlorochromate, and 3) stereoselective reduction of the 3-oxo compounds with *tert*-butylamine–borane complex. The results of analysis of the prepared 4β-hydroxylated bile acids with a diequatorial *trans*-glycol structure and their 3β-epimers by proton and carbon-13 nuclear magnetic resonance spectroscopies are briefly discussed along with the mass spectrometric properties.

Keywords bile acid; human fetal bile; 3α,4β-dihydroxy-5β-cholanoic acid; 3α,4β,7α-trihydroxy-5β-cholanoic acid; 3α,4β,12α-trihydroxy-5β-cholanoic acid; 3α,4β,7α,12α-tetrahydroxy-5β-cholanoic acid; mass spectrum; ^{1}H-NMR spectrum; ^{13}C-NMR spectrum; *cis*-dihydroxylation

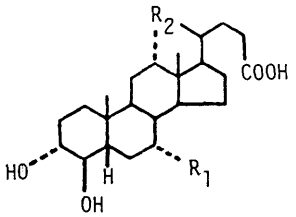
In recent years considerable attention has been focused on the difference between the fetal and adult pathways of bile acid synthesis from cholesterol.[2)] We have recently reported the isolation from human fetal bile of an unique bile acid, which accounted for 5—15% of the total biliary bile acids in early gestation.[3)] This novel bile acid was characterized by partial synthesis as 3α,4β,7α-trihydroxy-5β-cholanoic acid, with a diequatorial *trans*-glycol structure. In more recent work, two other 4β-hydroxylated bile acids, 3α,4β-dihydroxy- and 3α,4β,7α,12α-tetrahydroxy-5β-cholanoic acids, have been identified by gas chromatographic-mass spectrometric analysis in the meconium and feces of newborn infants.[3,4)] These findings indicate the existence of a previously unknown hepatic biotransformation pathway, namely that of 4β-hydroxylation.

For our series of studies on new and scarce bile acid metabolites, we required a moderate supply of the 4β-hydroxylated bile acids as authentic reference compounds. This paper describes the synthesis of 4β-hydroxylated derivatives (**1a—d**) of lithocholic, deoxycholic, chenodeoxycholic, and cholic acids and their corresponding 3β-epimers (**2a—d**), starting from their respective parent bile acid methyl esters (**3a—d**) (Chart 1).

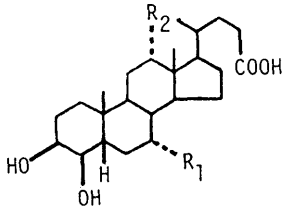
As outlined in Chart 2, the key intermediates in our work are the 3β,4β-dihydroxylated compounds (**6a** and **6e—g**), which were prepared in total yields of 64—38%. The procedures involve tosylation of **3a—d** with *p*-toluenesulfonyl chloride–pyridine[5)] and subsequent elimination of the 3-tosyloxy group in boiling 2,6-lutidine.[6-9)] Each dehydrotosylation reaction provided predominantly a single olefinic product, *i.e.*, the Δ^3 compounds (**5a–c**), except for **4d** which gave rise to a small amount (14%) of the Δ^2 isomer together with the desired **5d**. The Δ^3 structure was characterized by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy; **5a** and **5b** show multiplet signals at 5.33 ppm (4-H) and 5.59 ppm (3-H), while the corresponding protons in **5c** and **5d** occur at 5.70 ppm as a broad singlet ($W_{1/2}$, 3.0 Hz), probably due to the steric effect of an axial 7α-hydroxyl group. Prior to the subsequent reactions, free hydroxyl groups at positions C-7 and/or C-12 in **5b—d** were protected as the acetates **5e—g** by the usual acetic anhydride–pyridine method.

Treatment of the Δ^3 compounds (**5a** and **5e—g**) with *N*-methylmorpholine *N*-oxide in the presence of a catalytic amount of osmium tetroxide (OsO_4) in *tert*-butyl alcohol–tetrahydrofuran–water mixture (10 : 30 : 1, v/v)[10,11)] led to β-face *cis*-dihydroxylation.[12,13)] The hydroxylation reaction proceeded smoothly and sterically pure 3β,4β-dihydroxy intermediates (**6a** and **6e—g**) were formed in good isolated yields (78—85%) without detectable amounts of the 3α,4α-dihydroxy epimers.[12)] To obtain colorless products, neutral alumina was used as chromatographic adsorbent, which removed efficiently the dark brown contaminants. The assignment of the vicinal 3β,4β-glycol structure was based on the ^{1}H-NMR signals appearing at 3.98 ppm as a multiplet (equatorial 3α-H); and at 3.83 (**6a** and **6e**) or 4.09 (**6f** and **6g**) ppm as a broad multiplet (axial 4α-H). Usual alkaline hydrolysis of **6a** and **6e—g** afforded nearly quantitatively the 3β-epimers (**2a—d**) of 4β-hydroxylated bile acids.

To obtain the 4β-hydroxy-3-oxo compounds **9f** and **9g** from their respective diols, the most promising route seemed to be oxidation of their derivatives with a protected 4β-hydroxyl group. Since early studies on selective acylation of steroidal polyhydroxy compounds had shown that equatorial hydroxyls react to form cathylates while axial ones do not,[14)] we had expected that the 3β,4β-diols **6a** and **6e—g**,

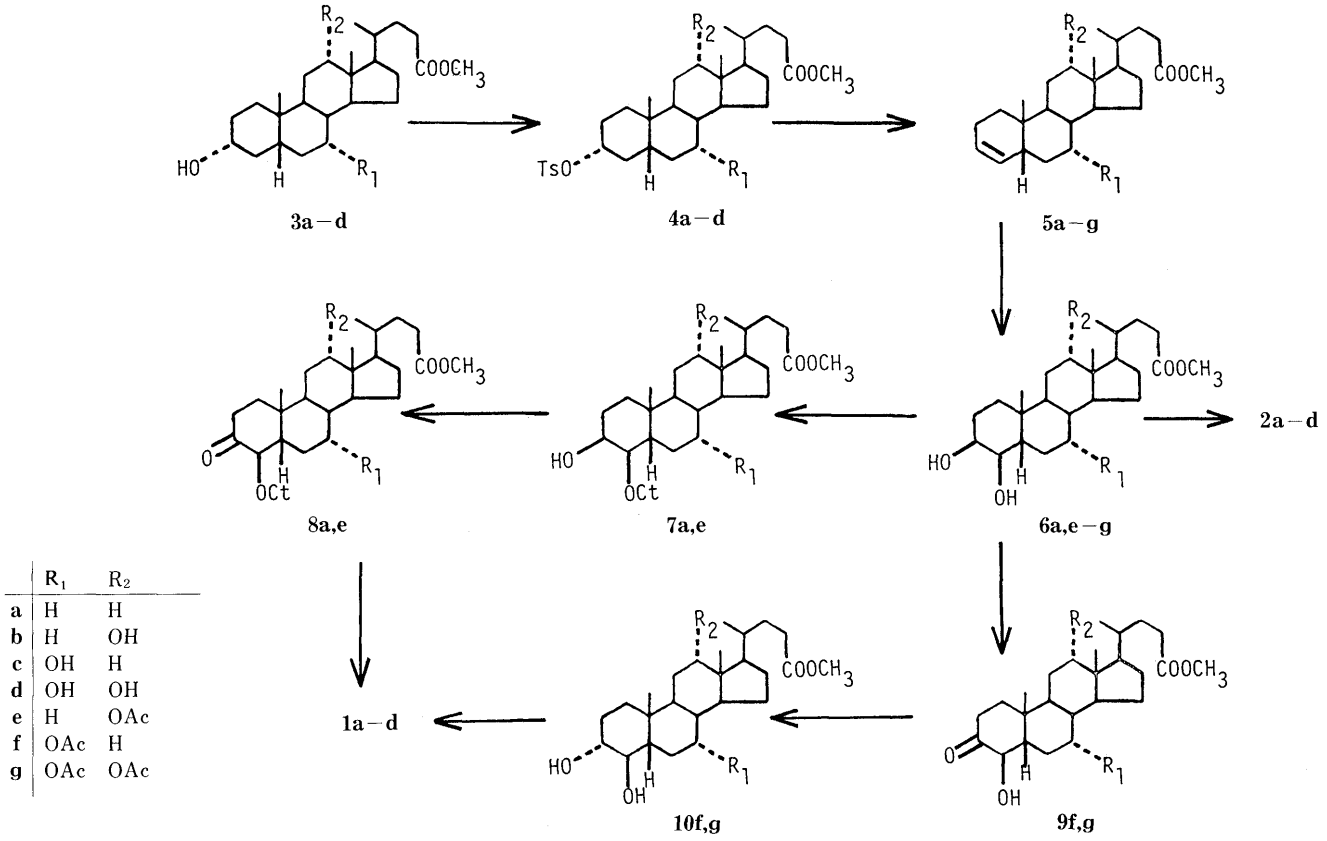


	R_1	R_2
1a	H	H
1b	H	OH
1c	OH	H
1d	OH	OH



	R_1	R_2
2a	H	H
2b	H	OH
2c	OH	H
2d	OH	OH

Chart 1. 4β-Hydroxylated Bile Acids and Their 3β-Epimers



	R_1	R_2
a	H	H
b	H	OH
c	OH	H
d	OH	OH
e	H	OAc
f	OAc	H
g	OAc	OAc

Chart 2. Synthetic Routes to 4β-Hydroxylated Bile Acids

all with equatorial groups at C-4, would react selectively to yield their 4-monocathylates. The latter derivatives would then be easily oxidized and subsequently hydrolyzed to the desired 4β-hydroxy-3-oxo compounds.

When the actual cathylation reactions with ethyl chloroformate in dioxane–pyridine as the reagent were carried out, the diols **6a** and **6e** were indeed satisfactorily monocathylated in 76 and 74% yields, respectively. The resulting 4-monocathylates **7a** and **7e** were oxidized with Jones reagent to give the corresponding 4β-cathyloxy-3-oxo esters **8a** and **8e** in nearly quantitative yields. The presence of the 4α-protons in **8a** and **8e** was confirmed by a doublet ^{1}H-NMR signal coupled with 5-H at 5.34 ppm (*J*, 11.7 Hz).

In contrast with the facile mono-cathylation of **6a** and **6e**, the diols **6f** and **6g**, both with additional 7α-acetoxy groups, under identical reaction conditions, yielded mixtures consisting of 3,4-dicathylates, 3- and 4-monocathylates and the starting diol in approximately equal parts.[3] The target 4β-monocathylates were isolated in only poor yields after careful chromatographic purification. The absence of selectivity in the cathylation reaction with **6f** and **6g** may be attributed to conformational distortion of ring A due to steric hindrance of the axial 7α-acetoxy group.[15]

Thus, in seeking a more efficient route to the 4β-hydroxy-3-oxo esters **9f** and **9g**, we investigated reactions which might selectively oxidize the hydroxyl group at C-3. Most oxidizing reagents such as Jones reagent are known to cleave 3,4-diols to seco acids.[12] Silver carbonate-Celite, a reagent which is used to oxidize selectively C-3 equatorial alcohols in numerous polyhydroxy bile acids and other steroids,[16,17] was unsuccessful when applied to the diols **6f** and **6g**. The desired mono-oxidation of **6f** and **6g** was finally achieved by direct reaction with pyridinium chlorochromate[18,19] to afford the 3-oxo derivatives **9f** and **9g** in yields of 59 and 51%, respectively. Each compound was characterized by the appearance of a doublet signal in the ^{1}H-NMR at 4.65-4.66 ppm (*J*, 11.7 Hz) due to the 4α-proton.

The final step of stereoselective reduction of the four ketones, **8a**, **8e**, **9f** and **9g**, to their corresponding C-3 equatorial alcohols initially appeared simply to require reduction by sodium borohydride, which normally reduces saturated 3-ketones stereoselectively to C-3 equatorial products.[20] However, with the 4β-cathyloxy-3-oxo ester **8f**, sodium borohydride unexpectedly yielded the desired 3α-hydroxy compound only as the minor product (18%)[3]; the predominant product was its epimer **7f**, probably due to shielding of the β-face of the molecule by the bulky adjacent 4β-substituent.

Ultimately, selective reduction to the 3α-hydroxy analogs was successful when the reagent used was the *tert*-butylamine–borane complex which we had previously found to exhibit high equatorial selectivity in the reduction of 6-and 12-oxo bile acids.[17,21] With this reagent the 4β-cathyloxy-3-oxo esters **8a** and **8e** underwent stereoselective reduction at C-3, and after hydrolysis of the cathyloxy groups, yielded the 3α,4β-diols **1a** and **1b** in 80 and 74% isolated yields, respectively. Similarly, reduction of the 4β-hydroxy-3-oxo esters **9f** and **9g** with the same reagent yielded the desired 3α,4β-dihydroxy esters **10f** and **10g** in 81 and 83% isolated yields. Small amounts of the 3β-epimers **6f** and **6g** were readily removed by chromatography on silica gel. Alkaline hydrolysis of **10f** and **10g** followed by

acidification afforded nearly quantitatively the 3α,4β-dihydroxy acids **1c** and **1d**.

Chemical evidence for the vicinal glycol structure, the stereochemical configuration of hydroxyls, and the purity of the 4β-hydroxylated bile acids (**1a—d**) and their 3β-epimers (**2a—d**) were further confirmed as their methyl

TABLE I. Relative Abundances of Fragment Ions for Methyl Ester Derivatives of 4β-Hydroxylated Bile Acids (**1a—d** and **2a—d**)

Fragment ion (*m/z*)	3α,4β-(OH)$_2$	3α,4β,12α-(OH)$_3$	3α,4β,7α-(OH)$_3$	3α,4β,7α,12α-(OH)$_4$	3β,4β-(OH)$_2$	3β,4β,12α-(OH)$_3$	3β,4β,7α-(OH)$_3$	3β,4β,7α,12α-(OH)$_4$
M^+	16	>1			76	>1	>1	
$(M-H_2O)^+$	100	9	25	4	100	4	100	29
$(M-2H_2O)^+$	23	9	100	21	26	1	39	65
$(M-3H_2O)^+$		5	14	25		1	7	24
[M−side chain(115)−H_2O]$^+$	3	100	15		5	100	19	
$(M-115-2H_2O)^+$	1	23	24	35	5	23	11	100
$(M-115-3H_2O)^+$		14	2	100		13	5	44
[M−115−part of ring D(27)]$^+$	18				60			
[M−115−ring D(42)]$^+$	21	2			33	2	1	1
$(M-115-27-2H_2O)^+$	2	1	3	12	6	1	8	54
$(M-115-27-3H_2O)^+$		1		32			1	14
$(M-115-42-H_2O)^+$	24	2	1	1	59	1	7	2
$(M-115-42-2H_2O)^+$	30	3	4	1	39	2	14	3
127	1	1	23	25	4		75	62

TABLE II. 500 MHz ^{1}H-NMR Spectral Data for Methyl Ester Derivatives of 4β-Hydroxylated Bile Acids (**1a—d** and **2a—d**)[a]

Bile acids	18-Me[b]	19-Me[b]	21-Me[c]	COOMe[b]	3-H[c]	4α-H[c]	7β-H[c]	12β-H[c]
3α,4β-(OH)$_2$	0.62	0.94	0.88 (d, 6.5)	3.64	3.38 (br m, 26.8)	3.70 (dd, 10.7 and 9.1)		
3α,4β,12α-(OH)$_3$	0.66	0.93	0.94 (d, 6.5)	3.64	3.37 (br m, 26.6)	3.73 (dd, 10.2 and 8.6)		3.95 (t, 2.6)
3α,4β,7α-(OH)$_3$	0.64	0.92	0.90 (d, 6.5)	3.64	3.25 (br m, 26.4)	4.12 (dd, 10.6 and 9.0)	3.87 (m, 6.8)	
3α,4β,7α,12α-(OH)$_4$	0.65	0.90	0.95 (d, 6.5)	3.64	3.25 (br m, 26.0)	4.12 (dd, 10.7 and 9.1)	3.88 (m, 7.5)	3.94 (t, 2.8)
3β,4β-(OH)$_2$	0.63	0.96	0.88 (d, 6.5)	3.64	3.99 (m, 8.5)	3.86 (dd, 11.3 and 3.0)		
3β,4β,12α-(OH)$_3$	0.67	0.95	0.94 (d, 6.5)	3.64	3.99 (m, 8.3)	3.89 (dd, 11.2 and 3.0)		3.96 (t, 2.4)
3β,4β,7α-(OH)$_3$	0.64	0.94	0.90 (d, 6.5)	3.64	3.95 (m, 8.1)	4.35 (dd, 11.1 and 3.4)	3.88 (m, 6.5)	
3β,4β,7α,12α-(OH)$_4$	0.68	0.93	0.95 (d, 6.0)	3.64	3.94 (m, 8.4)	4.36 (dd, 11.1 and 3.3)	3.90 (m, 6.9)	3.97 (t, 2.9)

a) In ppm down field from Me_4Si. *b*) Singlet. *c*) Values in parentheses refer to signal multiplicity and coupling constant (*J* in Hz) or width at half-height ($W_{1/2}$ in Hz): br m, broad multiplet; d, doublet; dd, double doublet; t, triplet.

TABLE III. ^{13}C-NMR Spectral Data for Methyl Ester Derivatives of 4β-Hydroxylated Bile Acids (**1a—d** and **2a—d**)[a]

Carbon	3α,4β-(OH)$_2$	3α,4β,12α-(OH)$_3$	3α,4β,7α-(OH)$_3$	3α,4β,7α,12α-(OH)$_4$	3β,4β-(OH)$_2$	3β,4β,12α-(OH)$_3$	3β,4β,7α-(OH)$_3$	3β,4β,7α,12α-(OH)$_4$
1	34.6	34.6	34.6	34.6	29.2	29.1	29.2	29.2
2	27.3	27.4	28.1	28.1	25.8[b]	25.7	25.9	25.6
3	76.7	76.7	75.9[b]	76.0[b]	68.4[c]	68.3[b]	69.5	69.7
4	72.7	72.5	75.5[b]	75.3[b]	69.5[c]	69.5[b]	71.4	71.1
5	48.7	48.5	47.9	47.9	44.0	43.9	42.9	42.7
6	20.9	20.8	27.5	27.5	21.1	20.6	28.0	28.6[b]
7	26.0	25.8	68.5	68.5	26.0[b]	25.7	68.6	68.9
8	35.7	35.9[b]	39.2	39.2	35.5	35.7	39.3	39.4
9	42.4	35.4[b]	34.9	28.1	41.6	34.9	34.0	27.4
10	37.0	36.4	37.1	36.8	36.6	36.1	36.9	36.6
11	20.9	28.7	20.7	28.1	20.7	28.9	20.9	28.0[b]
12	40.1	73.0	39.6	73.0	40.1	73.1	39.6	73.1
13	42.7	46.5	42.7	46.4	42.6	46.4	42.7	46.4
14	56.5	48.3	50.5	41.6	56.6	48.3	50.4	41.8
15	24.1	23.6	23.6	23.1	24.1	23.5	23.6	23.2
16	28.1	27.4	28.1	27.5	28.1	27.4	28.1	27.4
17	56.0	47.3	55.9	47.0	56.0	47.2	55.8	47.2
18	12.0	12.7	11.8	12.4	12.0	12.7	11.8	12.5
19	23.5	23.2	22.9	22.5	23.6	23.4	22.9	22.6
20	35.3	35.1	35.3	35.2	35.3	35.0	35.3	35.2
21	18.2	17.3	18.3	17.3	18.2	17.3	18.3	17.3
22	31.0	31.1	31.0	31.0	31.0	31.0	31.0	31.1
23	31.0	30.9	31.0	31.0	31.0	30.9	31.0	30.9
24	174.6	174.6	174.6	174.7	174.6	174.6	174.6	174.7
25	51.3	51.4	51.4	51.4	51.4	51.4	51.3	51.4

a) In ppm downfield from Me_4Si. *b, c*) Assignments in each column may be interchanged.

ester derivatives by mass and 500 MHz ^{1}H- and ^{13}C-NMR spectral data. The results are compiled in Tables I—III.

In the mass spectra (MS) of the 4β-hydroxylated bile acids, principal fragment ions arise from the loss of the side chain (SC), SC and part of ring D, and/or SC and ring D accompanied with the elimination of one to three molecules of water from molecular ion (M^+). In compounds having a 7α-hydroxyl group, a characteristic ion was observed at m/z 127; the origin of this unique ion is unclear.

In the 500 MHz ^{1}H-NMR spectra, axial 3β-H in **1a—d** appears at 3.25—3.38 ppm as a broad multiplet ($W_{1/2}$, 26.0—26.8 Hz), while the corresponding equatorial 3α-H in **2a—d** occurs at 3.94—3.99 ppm as a multiplet ($W_{1/2}$, 8.1—8.5 Hz) due to coupling with 2- and 4-H. On the other hand, axial 4α-H in **1a** and **1b** with a diequatorial *trans*-glycol structure resonates at 3.70—3.73 ppm as a double doublet with J values of 10.2—10.7 and 8.6—9.1 Hz due to coupling with 3- and 5-H; this proton signal is deshielded by 0.39—0.42 ppm and resonates at 4.12 ppm in **1c** and **1d** because of the steric hindrance of the 7α-hydroxyl group. The corresponding axial 4α-H in **2a** and **2b** and in **2c** and **2d**, which have an axial-equatorial *cis*-glycol structure, shows a double doublet at 3.86—3.89 and 4.35—4.36 ppm, respectively, with J values of 11.1—11.3 and 3.0—3.4 Hz. The remaining proton signals, equatorial 7β- and 12β-H, appear at 3.87—3.90 ppm as a multiplet ($W_{1/2}$, 6.5—7.5 Hz) and at 3.94—3.97 ppm as a triplet (J, 2.4—2.9 Hz) due to coupling with 6- and 8-H and 11-H, respectively.

The assignment of each carbon signal of **1a—d** and **2a—d** in the ^{13}C-NMR spectrum was made on the basis of procedures reported previously.[22] Signal assignment of the carbon atoms situated in close proximity to hydroxyl groups at C-3 and C-4 was based, to a large extent, on the work of VanAntwerp, *et al.*, who reported the effect of a wide range of 1,2- or 1,3-dihydroxy groups on the ^{13}C-NMR signals of dihydroxy steroids.[23] The shielding data of the α-carbon absorptions (3α-, 3β-, 4β-, 7α-, and 12α-OH) in the lower field region (68—77 ppm) are of particular importance in characterizing the number, position, and configuration of the hydroxyl groups. Since these sharp signals are unequivocally identified and separated completely from each other, the shielding data allow a straightforward identification of each compound as well as an estimation of its purity.

Experimental

Melting points (mp) were determined on a micro hot stage apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 1600 Series FTIR as KBr tablets. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL FX-90Q instrument with $CDCl_3$ containing 1% Me_4Si as the solvent, except where otherwise indicated; chemical shifts are expressed in δ (ppm) relative to Me_4Si. The high-resolution ^{1}H-NMR spectra were also recorded on a JEOL GSX-500 instrument at 500 MHz. MS were recorded on a JEOL JMS-01SG-2 mass spectrometer at 60 eV. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates (20 cm × 20 cm, 0.25 mm layer thickness; E. Merck AG) using hexane–EtOAc–acetic acid mixture (80 : 20 : 1—10 : 40 : 2, v/v) as the developing solvent. All compounds were dried by azeotropic distillation before use in reactions.

General Procedure for the Hydrolysis of Methyl Esters to Free Acids The ester (300 mg) was refluxed in 5% methanolic KOH for 1 h or in 10% methanolic KOH for 10 h in the case of compounds having a 12α-acetoxy group. Most of the solvent was evaporated off, and the residue was dissolved in water. This solution was cooled in an ice-bath, and acidified with 10% H_2SO_4 with stirring. The precipitate was collected by filtration, washed with water, and recrystallized from an appropriate solvent.

General Procedure for the Esterification of Free Acids to Methyl Esters *p*-Toluenesulfonic acid (30 mg) was added to the free acid (300 mg) in methanol (9 ml), and the mixture was allowed to stand overnight at room temperature. Most of methanol was evaporated off, and the residue was extracted with CH_2Cl_2. The organic extract was washed with successively with water, 5% $NaHCO_3$, and water, dried with Drierite, and evaporated to give the corresponding ester, which was crystallized from an appropriate solvent.

Methyl 3α-Tosyloxy-5β-cholanoate (4a) Tosylation of **3a** was carried out by the usual *p*-toluenesulfonyl chloride–pyridine method described in a previous paper.[9] Crystallization of the oily product from MeOH gave **4a** (79%) as colorless crystals, mp 115—117 °C (lit. mp 110—112 °C[24] and mp 119—121 °C[25]). IR ν_{max} cm^{-1}: 1734 (C=O), 1360, 1172 (SO_2). ^{1}H-NMR δ: 0.62 (3H, s, 18-Me), 0.88 (3H, s, 19-Me), 0.91 (3H, d, J=4.5 Hz, 21-Me), 2.44 (3H, s, phenyl Me), 3.66 (3H, s, COOMe), 4.47 (1H, br m, 3-H), 7.31 and 7.80 (each 2H, d, J=8.1 Hz, *p*-disubstituted phenyl).

Methyl 12α-Hydroxy-3α-tosyloxy-5β-cholanoate (4b) Prepared from **3b** by the tosylation procedure described above. Recrystallization of the product from benzene–hexane gave **4b** (86%) as colorless prisms, mp 145—147 °C (lit, mp 149—150 °C[6]). IR ν_{max} cm^{-1}: 1719 (C=O), 3519, 1020 (OH), 1353, 1176 (SO_2). ^{1}H-NMR δ: 0.66 (3H, s, 18-Me), 0.87 (3H, s, 19-Me), 0.96 (3H, d, J=6.3 Hz, 21-Me), 2.44 (3H, s, phenyl Me), 3.66 (3H, s, COOMe), 3.95 (1H, m, 12-H), 4.48 (1H, br m, 3-H), 7.31 and 7.79 (each 2H, d, J=9.0 Hz, *p*-disubstituted phenyl).

Methyl 7α-Hydroxy-3α-tosyloxy-5β-cholanoate (4c) Prepared from **3c** by the tosylation procedure described above. Recrystallization of the product from benzene–hexane gave **4c** (86%) as colorless thin plates, mp 135—137 °C (lit. mp 128.5—129.5 °C[8] and mp 129—131 °C[26]). IR ν_{max} cm^{-1}: 1737 (C=O), 3555, 1005, 985 (OH), 1353, 1168 (SO_2). ^{1}H-NMR δ: 0.64 (3H, s, 18-Me), 0.87 (3H, s, 19-Me), 2.44 (3H, s, phenyl Me), 3.66 (3H,s, COOMe), 3.80 (1H, m, 7-H), 4.29 (1H, br m, 3-H), 7.31 and 7.79 (each 2H, d, J=9.0 Hz, *p*-disubstituted phenyl).

Methyl 7α,12α-Dihydroxy-3α-tosyloxy-5β-cholanoate (4d) Prepared from **3d** by the tosylation procedure described above. Recrystallization of the product from aqueous MeOH gave **4d** (88%) as colorless crystals, mp 132—133 °C (lit. mp 133—134 °C[8] and mp 132.5—133.5 °C[9]). IR ν_{max} cm^{-1}: 1716 (C=O), 3623, 1020, 983 (OH), 1353, 1176 (SO_2). ^{1}H-NMR δ: 0.67 (3H, s, 18-Me), 0.86 (3H, s, 19-Me), 0.97 (3H, d, J=4.5 Hz, 21-Me), 2.44 (3H, s, phenyl Me), 3.66 (3H, s, COOMe), 3.84 (1H, m, 7-H), 3.95 (1H, m, 12-H), 4.34 (1H, br m, 3-H), 7.31 and 7.79 (each 2H, d, J=9.0 Hz, *p*-disubstituted phenyl).

Methyl 5β-Chol-3-enoate (5a) A solution of **4a** (5.0 g) in 2,6-lutidine (50 ml) was refluxed under N_2 for 1 h. Most of the 2,6-lutidine was evaporated off under reduced pressure, and the residue was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with 10% HCl and water, dried over Drierite, and evaporated. The residue, when treated with aqueous acetone, afforded **5a** (2.93 g, 86%) as colorless thin plates, mp 71—72 °C (lit. mp 74.5—75 °C[5] and mp 75—77 °C[23]). IR ν_{max} cm^{-1}: 1733 (C=O). ^{1}H-NMR δ: 0.66 (3H, s, 18-Me), 0.91 (3H, d, J=7.2 Hz, 21-Me), 0.95 (3H, s, 19-Me), 3.66 (3H, s, COOMe), 5.33 (1H, m, 4-H), 5.58 (1H, m, 3-H).

Methyl 12α-Hydroxy-5β-chol-3-enoate (5b) Prepared from **4b** by the dehydrotosylation procedure as described for the preparation of **5a** and crystallized from benzene–hexane as colorless needles (84%), mp 110—111 °C (lit. mp 111—112 °C[5]). IR ν_{max} cm^{-1}: 1740 (C=O), 3202, 1030, 975 (OH). ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 0.94 (3H, s, 19-Me), 0.97 (3H, d, J=5.4 Hz, 21-Me), 3.66 (4H, s, COOMe and 7-H), 3.97 (1H, m, 12-H), 5.33 (1H, m, 4-H), 5.60 (1H, m, 3-H).

Methyl 7α-Hydroxy-5β-chol-3-enoate (5c) Prepared from **4c** by the dehydrotosylation procedure as described for the preparation of **5a** and crystallized from aqueous MeOH as colorless prisms (89%), mp 110-112 °C (lit. mp 117—120 °C[8]). IR ν_{max} cm^{-1}: 1737 (C=O), 3553, 1032 (OH). ^{1}H-NMR δ: 0.67 (3H, s, 18-Me), 0.92 (3H, d, J=6.3 Hz, 21-Me), 0.97 (3H, s, 19-Me), 3.66 (3H, s, COOMe), 3.71 (1H, br m, 7-H), 5.70 (2H, s, $W_{1/2}$=3.0 Hz, 3- and 4-H).

Methyl 7α,12α-Dihydroxy-5β-chol-3-enoate (5d) **4d** (10.0 g), treated with 2,6-lutidine and processed as described for the preparation of **5a**, yielded 6.88 g of the crude product, which consisted of a mixture of two components as judged by TLC. Chromatography of the product on silica gel (350 g) separated the two components. The first fraction eluted with benzene–EtOAc (4 : 6, v/v) gave 0.98 g (14%) of the minor component, which was crystallized from benzene–hexane and characterized as methyl 7α,12α-dihydroxy-5β-chol-2-enoate, mp 157—159 °C. IR ν_{max} cm^{-1}: 1720 (C=O), 3510, 1035, 972 (OH). ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 0.96 (3H,

s, 19-Me), 0.99 (3H, d, J=4.5 Hz, 21-Me), 3.66 (3H, s, COOMe), 3.93 (2H, m, 7- and 12-H), 5.38—5.71 (2H, m, 2- and 3-H). *Anal.* Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.38; H, 9.96.

The second fraction eluted with benzene–EtOAc (2:8, v/v) gave 4.12 g (59%) of the main component, which was identified as the desired **5d** and crystallized as colorless needles from benzene–hexane, mp 136—137 °C (lit. mp 121—123.5 °C[9]). IR ν_{max} cm^{-1}: 1743 (C=O), 3405, 990 (OH). ^{1}H-NMR δ: 0.71 (3H, s, 18-Me), 0.96 (3H, s, 19-Me), 0.99 (3H, d, J=4.5 Hz, 21-Me), 3.66 (3H, s, COOMe), 3.70 (1H, br m, 7-H), 3.98 (1H, m, 12-H), 5.70 (2H, s, $W_{1/2}$=3.0 Hz, 3- and 4-H). *Anal.* Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.02; H, 10.04.

Methyl 12α-Acetoxy-5β-chol-3-enoate (5e) A mixture of **5b** (8.0 g) and acetic anhydride (10 ml) in dry pyridine (10 ml) was refluxed for 2 h. The reaction mixture was poured onto ice-water, and the precipitate was collected by filtration and washed with water. Recrystallization from aqueous MeOH gave **5e** (8.38 g, 95%) as colorless thin plates, mp 105—107 °C. IR ν_{max} cm^{-1}: 1736 (C=O), 1250, 1027 (acetate). ^{1}H-NMR δ: 0.74 (3H, s, 18-Me), 0.80 (3H, d, J=5.4 Hz, 21-Me), 0.92 (3H, s, 19-Me), 2.05 (3H, s, 12-OCOMe), 3.66 (3H, s, COOMe), 5.08 (1H, m, 12-H), 5.33 (1H, m, 4-H), 5.58 (1H, m, 3-H). *Anal.* Calcd for $C_{27}H_{42}O_4 \cdot 1/4H_2O$: C, 74.53; H, 9.84. Found: C, 74.81; H, 9.96.

Methyl 7α-Acetoxy-5β-chol-3-enoate (5f) Prepared from **5c** (8.0 g) by the acetylation procedure described above. The reaction mixture was diluted with water and the product was extracted with CH_2Cl_2. The combined extract was washed with 10% HCl and water, dried over Drierite, and evaporated. The oily product (8.79 g, 99%), although apparently homogeneous on TLC and ^{1}H-NMR analyses, could not be crystallized. IR ν_{max} cm^{-1}: 1724 (C=O), 1258 (acetate). ^{1}H-NMR δ: 0.66 (3H, s, 18-Me), 0.92 (3H, d, J=5.4 Hz, 21-Me), 0.97 (3H, s, 19-Me), 1.99 (3H, s, 7-OCOMe), 3.66 (3H, s, COOMe), 4.79 (1H, m, 7-H), 5.29 (1H, m, 4-H), 5.48 (1H, m, 3-H). High-resolution MS: 430.3083 (M^+, $C_{27}H_{42}O_4$ requires 430.3083).

Methyl 7α,12α-Diacetoxy-5β-chol-3-enoate (5g) Prepared from **5d** (8.0 g) by the acetylation procedure described above. Recrystallization of the oily product from aqueous MeOH gave **5g** (8.97 g, 93%) as colorless needles, mp 115—116 °C. IR ν_{max} cm^{-1}: 1732 (C=O), 1252, 1026 (acetate). ^{1}H-NMR δ: 0.74 (3H, s, 18-Me), 0.82 (3H, d, J=5.4 Hz, 21-Me), 0.95 (3H, s, 19-Me), 2.01 and 2.08 (each 3H, s, 7- and 12-OCOMe), 3.66 (3H, s, COOMe), 4.83 (1H, m, 7-H), 5.08 (1H, m, 12-H), 4.96 (1H, m, 4-H), 5.48 (1H, m, 3-H). *Anal.* Calcd for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.33; H, 9.27.

Methyl 3β,4β-Dihydroxy-5β-cholanoate (6a) OsO_4 (100 mg) and *N*-methylmorpholine *N*-oxide (3.75 g) were added to a solution of **5a** (5.0 g) dissolved in *tert*-butyl alcohol–tetrahydrofuran–water (50 ml; 10:30:1, v/v/v), and the mixture was allowed to stand overnight at room temperature. The dark brown solution was poured into water, and extracted with CH_2Cl_2. The extract was washed with 10% HCl, 5% $NaHCO_3$, and water, dried over Drierite, and evaporated. The oily residue was chromatographed on neutral alumina (activity III, ratio, 25:1). Elution with benzene–EtOAc (2:8, v/v) and recrystallization of the eluate from aqueous MeOH gave **6a** (4.42 g, 81%) as colorless crystals, mp 131—133 °C (lit. mp 128—129 °C[12]). IR ν_{max} cm^{-1}: 1732 (C=O), 3382, 1030, 983 (OH). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.90 (3H, d, J=5.4 Hz, 21-Me), 0.98 (3H, s, 19-Me), 3.66 (3H, s, COOMe), 3.82 (1H, br m, 4-H), 3.97 (1H, m, 3-H). *Anal.* Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 73.53; H, 10.28.

Methyl 12α-Acetoxy-3β,4β-dihydroxy-5β-cholanoate (6e) *cis*-Dihydroxylation of **5e** (5.0 g) was carried out by the catalytic OsO_4 method as described for the preparation of **6a**. After column chromatographic purification, the eluate was recrystallized from aqueous MeOH to give **6e** (4.22 g, 78%) as colorless needles, mp 174—175 °C. IR ν_{max} cm^{-1}: 1741 (C=O), 3360 (OH), 1249, 1029 (acetate). ^{1}H-NMR δ: 0.73 (3H, s, 18-Me), 0.80 (3H, d, J=5.4 Hz, 21-Me), 0.96 (3H, s, 19-Me), 2.07 (3H, s, 12-OCOMe), 3.66 (3H, s, COOMe), 3.84 (1H, br m, 4-H), 3.98 (1H, m, 3-H), 5.07 (1H, m, 12-H). *Anal.* Calcd for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.51: H, 9.72.

Methyl 7α-Acetoxy-3β,4β-dihydroxy-5β-cholanoate (6f) *cis*-Dihydroxylation of **5f** (5.0 g) was carried out by the catalytic OsO_4 method as described for the preparation of **6a**. After column chromatographic purification, the eluate was recrystallized from aqueous MeOH to give **6f** (4.58 g, 85%) as colorless thin plates, mp 139—140 °C. IR ν_{max} cm^{-1}: 1736 (C=O), 3412 (OH), 1244, 1024 (acetate). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.92 (3H, d, J=6.3 Hz, 21-Me), 0.98 (3H, s, 19-Me), 2.06 (3H, s, 7-OCOMe), 3.66 (3H, s, COOMe), 3.99 (1H, m, 3-H), 4.11 (1H, br m, 4-H), 4.91 (1H, m, 7-H). *Anal.* Calcd for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.87; H, 9.53.

Methyl 7α,12α-Diacetoxy-3β,4β-dihydroxy-5β-cholanoate (6g) *cis*-Dihydroxylation of **5g** (5.0 g) was carried out by the catalytic OsO_4 method as described for the preparation of **6a**. After column chromatographic purification, the eluate was recrystallized from aqueous MeOH to give **6g** (4.21 g, 79%) as colorless crystals, mp 74—76 °C. IR ν_{max} cm^{-1}: 1736 (C=O), 3504, 965 (OH), 1244, 1026 (acetate). ^{1}H-NMR δ: 0.74 (3H, s, 18-Me), 0.81 (3H, d, J=5.4 Hz, 21-Me), 0.96 (3H, s, 19-Me), 2.10 (6H, s, 7- and 12-OCOMe), 3.66 (3H, s, COOMe), 3.98 (1H, m, 3-H), 4.07 (1H, br m, 4-H), 4.94 (1H, m, 7-H), 5.08 (1H, m, 12-H). *Anal.* Calcd for $C_{29}H_{46}O_8$: C, 66.64; H, 8.87. Found: C, 66.34; H, 9.06.

3β,4β-Dihydroxy-5β-cholanoic Acid (2a) **6a** was hydrolyzed by the usual method. Recrystallization of the product from aqueous MeOH gave **2a** as colorless thin plates, mp 189—191 °C (lit. mp 191—192 °C[12]). IR ν_{max} cm^{-1}: 1708 (C=O), 3412, 1028 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.65 (3H, s, 18-Me), 0.92 (3H, d, J=6.3 Hz, 21-Me), 0.96 (3H, s, 19-Me), 3.71 (1H, br m, 4-H), 3.93 (1H, m, 3-H). *Anal.* Calcd for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.68; H, 10.04.

3β,4β,12α-Trihydroxy-5β-cholanoic Acid (2b) Prepared from **6e** by the usual alkaline hydrolysis. Recrystallization of the product from aqueous MeOH gave **2b** as colorless needles, mp 202—203 °C. IR ν_{max} cm^{-1}: 1705 (C=O), 3412, 1029 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.66 (3H, s, 18-Me), 0.93 (3H, s, 19-Me), 0.97 (3H, d, J=6.3 Hz, 21-Me), 3.76 (1H, br m, 4-H), 3.90 (2H, m, 3- and 12-H). *Anal.* Calcd for $C_{24}H_{40}O_5 \cdot 1/4H_2O$: C, 69.78; H, 9.88. Found: C, 69.68; H, 9.70.

Esterification of **2b** by the general method and recrystallization from acetone–hexane gave the corresponding methyl ester, mp 189—190 °C. IR ν_{max} cm^{-1}: 1739 (C=O), 3513, 1028 (OH). ^{1}H-NMR δ: 0.69 (3H, s, 18-Me), 0.97 (3H, s, 19-Me), 3.66 (3H, s, COOMe), 3.84 (1H, br m, 4-H), 3.99 (2H, m, 3- and 12-H). *Anal.* Calcd for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02. Found: C, 71.03; H, 9.99.

3β,4β,7α-Trihydroxy-5β-cholanoic Acid (2c) Prepared from **6f** by the usual hydrolysis method. Recrystallization of the product from aqueous MeOH gave **2c** as colorless thin plates, mp 212—214 °C. IR ν_{max} cm^{-1}: 1712 (C=O), 3372 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.65 (3H, s, 18-Me), 0.92 (3H, d, J=5.4 Hz, 21-Me), 0.94 (3H, s, 19-Me), 3.87 (2H, m, 3- and 7-H), 4.33 (1H, br m, 4-H). *Anal.* Calcd for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.62; H, 9.97.

Esterification of **2c** by the general method and recrystallization from aqueous MeOH gave the corresponding methyl ester as colorless needles, mp 203—205 °C. IR ν_{max} cm^{-1}: 1737 (C=O), 3386, 1025, 1008, 978 (OH). ^{1}H-NMR δ: 0.67 (3H, s, 18-Me), 0.93 (3H, d, J=6.3 Hz, 21-Me), 0.96 (3H, s, 19-Me), 3.66 (3H, s, COOMe), 3.93 (2H, m, 3- and 7-H), 4.36 (1H, br m, 4-H). *Anal.* Calcd for $C_{25}H_{42}O_5$: C, 71.05; H, 10.02. Found: C, 71.34; H, 10.12.

3β,4β,7α,12α-Tetrahydroxy-5β-cholanoic Acid (2d) Prepared from **6g** by the usual hydrolysis method. Recrystallization of the product from aqueous MeOH gave **2d** as colorless needles, mp 148—149 °C. IR ν_{max} cm^{-1}: 1708 (C=O), 3385, 1023 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.68 (3H, s, 18-Me), 0.94 (3H, s, 19-Me), 0.98 (3H, d, J=6.3 Hz, 21-Me), 3.93 (3H, m, 3-, 7- and 12-H), 4.36 (1H, br m, 4-H). *Anal.* Calcd for $C_{24}H_{40}O_6$: C, 67.89; H, 9.50. Found: C, 67.89; H, 9.62.

Esterification of **2d** by the general method and recrystallization from aqueous MeOH gave the corresponding methyl ester as colorless needles, mp 216—218 °C. IR ν_{max} cm^{-1}: 1720 (C=O), 3386, 1023, 981, 964 (OH). ^{1}H-NMR δ: 0.69 (3H, s, 18-Me), 0.95 (3H, s, 19-Me), 0.98 (3H, d, J=4.5 Hz, 21-Me), 3.66 (3H, s, COOMe), 3.95 (3H, m, 3-, 7- and 12-H), 4.32 (1H, br m, 4-H). *Anal.* Calcd for $C_{25}H_{42}O_6 \cdot 1/4H_2O$: C, 67.76; H, 9.67. Found: C, 67.85; H, 9.61.

Methyl 4β-Cathyloxy-3β-hydroxy-5β-cholanoate (7a) Ethyl chloroformate (1.33 g) was added dropwise to a solution of **6a** (3.3 g) in dioxane (50 ml) and dry pyridine (8 ml). The mixture was stirred overnight at room temperature, poured into water, and extracted with CH_2Cl_2. The organic layer was washed with 10% HCl and water, dried over Drierite, and evaporated. The oily residue was chromatographed on a column of silica gel (ratio, 30:1). Elution with benzene–EtOAc mixture provided three well-separated fractions. The less polar fraction eluted with benzene–EtOAc (9:1, v/v) was characterized as methyl 3β,4β-dicathyloxy-5β-cholanoate, which, although homogeneous according to TLC and ^{1}H-NMR analyses, resisted crystallization (0.36 g, 8%). IR ν_{max} cm^{-1}: 1738 (C=O), 1285 (=C–O). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.91 (3H, d, J=5.4 Hz, 21-Me), 1.02 (3H, s, 19-Me), 1.30 (6H, t, J=7.2 Hz, 3- and 4-OCOOCH$_2$CH$_3$), 3.66 (3H, s, COOMe), 4.19 (4H, q, J=7.2 Hz, 3- and 4-OCOOCH$_2$CH$_3$), 5.06 (1H, br m, 4-H), 5.23 (1H, m, 3-H). High-resolution MS: 550.3470 (M^+, $C_{31}H_{50}O_8$ requires 550.3506).

The second fraction eluted with benzene–EtOAc (8:2, v/v) gave the

main component, which was identified as the desired **7a**, and resisted crystallization (2.96 g, 76%). IR ν_{max} cm^{-1}: 1741 (C=O), 1268 (=C–O), 3566, 1011 (OH). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.91 (3H, d, J=5.4 Hz, 21-Me), 1.01 (3H, s, 19-Me), 1.32 (3H, t, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 3.66 (3H, s, COOMe), 4.13 (1H, m, 3-H), 4.20 (2H, q, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 5.08 (1H, dd, J=11.7 and 2.7 Hz, 4-H). High-resolution MS: 478.3259 (M$^+$, $C_{28}H_{46}O_6$ requires 478.3294).

The most polar fraction eluted with benzene–EtOAc (1:1, v/v) afforded the starting compound **6a** (0.17 g, 5%).

Methyl 12α-Acetoxy-4β-cathyloxy-3β-hydroxy-5β-cholanoate (7e) 6e (3.5 g) was treated with ethyl chloroformate and processed as described above. The oily product was chromatographed on a column of silica gel (ratio, 30:1). Elution with benzene–EtOAc (8:2, v/v) gave a homogeneous oil (0.38 g, 8%) which was characterized as methyl 12α-acetoxy-3β,4β-dicathyloxy-5β-cholanoate. IR ν_{max} cm^{-1}: 1734 (C=O), 1274 (=C–O). ^{1}H-NMR δ: 0.73 (3H, s, 18-Me), 0.80 (3H, d, J=5.4 Hz, 21-Me), 1.00 (3H, s, 19-Me), 1.31 (6H, t, J=7.2 Hz, 3- and 4-OCOOCH$_2$CH$_3$), 2.11 (3H, s, 12-OCOMe), 3.66 (3H, s, COOMe), 4.18 and 4.21 (each 2H, q, J=7.2 Hz, 3- and 4-OCOOCH$_2$CH$_3$), 5.08 (2H, br m, 4- and 12-H), 5.24 (1H, m, 3-H). High-resolution MS: 608.3582 (M$^+$, $C_{33}H_{52}O_{10}$ requires 608.3561).

Further elution with benzene–EtOAc (7:3, v/v) gave a homogeneous oil (2.98 g, 74%) which was identified as the desired **7e**. IR ν_{max} cm^{-1}: 1738 (C=O), 1260 (=C–O), 3545, 1024, 973 (OH). ^{1}H-NMR δ: 0.72 (3H, s, 18-Me), 0.80 (3H, d, J=5.4 Hz, 21-Me), 0.98 (3H, s, 19-Me), 1.32 (3H, t, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 2.10 (3H, s, 12-OCOMe), 3.66 (3H, s, COOMe), 4.13 (1H, m, 3-H), 4.21 (2H, q, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 5.07 (2H, br m, 4- and 12-H). High-resolution MS: 536.3376 (M$^+$, $C_{30}H_{48}O_8$ requires 536.3350).

The most polar fraction eluted with benzene–EtOAc (4:6, v/v) afforded the starting compound **6e** (0.29 g, 8%).

Methyl 4β-Cathyloxy-3-oxo-5β-cholanoate (8a) Jones reagent (5 ml) was added dropwise to a stirred solution of **6a** (2.0 g) in acetone (20 ml) under 10 °C, and then the mixture was further stirred for 30 min at room temperature. MeOH (4 ml) was added, and the reaction product was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, dried over Drierite, and evaporated. Recrystallization of the product from acetone-hexane afforded **8a** (1.82 g, 91%) as colorless prisms. mp 109—111 °C. IR ν_{max} cm^{-1}: 1743 (C=O), 1274 (=C–O). ^{1}H-NMR δ: 0.69 (3H, s, 18-Me) 0.93 (3H, d, J=6.3 Hz, 21-Me), 1.06 (3H, s, 19-Me), 1.34 (3H, t, J= 7.2 Hz, 4-OCOOCH$_2$CH$_3$), 3.66 (3H, s, COOMe), 4.23 (2H, q, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 5.34 (1H, d, J=11.7 Hz, 4-H). *Anal.* Calcd for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found: C, 70.39; H, 9.59.

Methyl 12α-Acetoxy-4β-cathyloxy-3-oxo-5β-cholanoate (8e) Oxidation of **7e** (2.0 g) was carried out with Jones reagent as described for the preparation of **8a**. Recrystallization of the product from aqueous MeOH gave **8e** (1.84 g, 92%) as colorless crystals, mp 165—166 °C. IR ν_{max} cm^{-1}: 1732 (C=O), 1274 (=C–O), 1032 (acetate). ^{1}H-NMR δ: 0.76 (3H, s, 18-Me), 0.80 (3H, d, J=6.3 Hz, 21-Me), 1.04 (3H, s, 19-Me), 1.35 (3H, t, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 2.12 (3H, s, 7-OCOMe), 3.66 (3H, s, COOMe), 4.25 (2H, q, J=7.2 Hz, 4-OCOOCH$_2$CH$_3$), 5.12 (1H, m, 12-H), 5.34 (1H, d, J=11.7 Hz, 4-H). *Anal.* Calcd for $C_{30}H_{46}O_8$: C, 67.39; H, 8.67. Found: C, 67.41; H, 8.81.

Methyl 7α-Acetoxy-4β-hydroxy-3-oxo-5β-cholanoate (9f) A suspension of **6f** (1.0 g), pyridinium chlorochromate (1.0 g), and powdered sodium acetate (0.5 g) in CH_2Cl_2 (100 ml) was stirred at room temperature for 30 min. The precipitated solid was filtered off, and the mother liquor was washed with 5% $NaHCO_3$ and water, dried over Drierite, and evaporated. The dark brown residue was chromatographed on a column of silica gel (ratio, 40:1). Elution with benzene–EtOAc (7:3, v/v) and recrystallization of the eluate from acetone–hexane gave **9f** (586 mg, 59%) as colorless thin plates, mp 162—164 °C. IR ν_{max} cm^{-1}: 1735 (C=O), 3481, 1017, 969 (OH), 1240, 1071 (acetate). ^{1}H-NMR δ: 0.70 (3H, s, 18-Me), 0.94 (3H, d, J=6.3 Hz, 21-Me), 1.03 (3H, s, 19-Me), 2.04 (3H, s, OCOMe), 3.66 (3H, s, COOMe), 4.65 (1H, d, J=11.7 Hz, 4-H), 5.02 (1H, m, 7-H). *Anal.* Calcd for $C_{27}H_{42}O_6 \cdot 1/4H_2O$: C, 69.42; H, 9.17. Found: C, 69.48; H, 9.29.

Methyl 7α,12α-Diacetoxy-4β-hydroxy-3-oxo-5β-cholanoate (9g) 6g (1.0 g) was oxidized with pyridinium chlorochromate and processed as described for the preparation of **9f**. Treatment of the crude product by chromatographic purification on silica gel (ratio, 40:1) eluting with benzene–EtOAc (7:3, v/v) and recrystallization of the major product from acetone–hexane gave **9g** (510 mg, 51%) as colorless prisms, mp 194—196 °C. IR ν_{max} cm^{-1}: 1740 (C=O), 3519, 1030, 994 (OH), 1248, 1080 (acetate). ^{1}H-NMR δ: 0.78 (3H, s, 18-Me), 0.82 (3H, d, J=6.3 Hz, 21-Me), 1.01 (3H, s, 19-Me), 2.08 and 2.11 (each 3H, s, 7- and 12-OCOMe), 3.66 (3H, s, COOMe), 4.66 (1H, d, J=11.7 Hz, 4-H), 5.08 (2H, m, 7- and 12-H). *Anal.* Calcd for $C_{29}H_{44}O_8$: C, 66.90; H, 8.52. Found: C, 67.14; H, 8.71.

Methyl 7α-Acetoxy-3α,4β-dihydroxy-5β-cholanoate (10f) *tert*-Butylamine–borane complex (95 mg) was added to a stirred solution of **9f** (200 mg) in CH_2Cl_2 (12 ml). The mixture was allowed to stand at room temperature for 2 h and then acidified with 10% HCl. The CH_2Cl_2 layer was washed with 5% $NaHCO_3$ and water, dried over Drierite, and evaporated. The oily residue was chromatographed on a column of silica gel (ratio, 40:1). Elution with benzene–EtOAc (6:4, v/v) provided two well-separated fractions. The less polar fraction (15 mg, 7%) was identified as **6f** by TLC and ^{1}H-NMR comparisons.

The more polar fraction (167 mg, 83%) was recystallized from aqueous MeOH to give the desired **10f** as colorless needles, mp 87—88 °C. IR ν_{max} cm^{-1}: 1737 (C=O), 3413, 1020, 966 (OH), 1249, 1068 (acetate). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.92 (3H, d, J=6.3 Hz, 21-Me), 0.96 (3H, s, 19-Me), 2.07 (3H, s, 7-OCOMe), 3.28 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.86 (1H, br m, 4-H), 4.90 (1H, m, 7-H). *Anal.* Calcd for $C_{27}H_{44}O_6 \cdot 1/4H_2O$: C, 69.12; H, 9.56. Found: C, 69.04; H, 9.39.

Methyl 7α,12α-Diacetoxy-3α,4β-dihydroxy-5β-cholanoate (10g) 9g (400 mg) was reduced with *tert*-butylamine–borane complex and processed as described for the preparation of **10f**. The crude product was chromatographed on a column of silica gel (ratio, 40:1). Elution with benzene–EtOAc (1:1, v/v) gave **10g** (324 mg, 81%) as the major product. The ester, although apparently homogeneous on TLC and ^{1}H-NMR analyses, could not be crystallized. IR ν_{max} cm^{-1}: 1736 (C=O), 3479, 965 (OH), 1244, 1025 (acetate). ^{1}H-NMR δ: 0.73 (3H, s, 18-Me), 0.81 (3H, d, J=5.4 Hz, 21-Me), 0.95 (3H, s, 19-Me), 2.10 and 2.12 (each 3H, s, 7- and 12-OCOMe), 3.25 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.89 (1H, br m, 4-H), 4.95 (1H, m, 7-H), 5.07 (1H, m, 12-H). High-resolution MS: 522.3196 (M$^+$, $C_{29}H_{46}O_8$ requires 522.3193).

3α,4β-Dihydroxy-5β-cholanoic Acid (1a) Prepared from **8a** (200 mg) by reduction with *tert*-butylamine–borane complex followed by alkaline hydrolysis. Several recrystallizations of the crude product from aqueous acetone gave the desired **1a** (122 mg, 74%) as colorless needles, partially melting at 114 °C, and melting at 156—158 °C. IR ν_{max} cm^{-1}: 1703 (C=O), 3456, 1019 (OH). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.93 (3H, d, J=6.3 Hz, 21-Me), 0.96 (3H, s, 19-Me), 3.41 (1H, br m, 3-H), 3.68 (1H, br m, 4-H). *Anal.* Calcd for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.37; H, 10.37.

The corresponding methyl ester, obtained by the usual method, crystallized from aqueous MeOH as colorless needles, mp 174—175 °C. IR ν_{max} cm^{-1}: 1741 (C=O), 3345, 1017 (OH). ^{1}H-NMR δ: 0.65 (3H, s, 18-Me), 0.91 (3H, d, J=6.3 Hz, 21-Me), 0.96 (3H, s, 19-Me), 3.41 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.75 (1H, br m, 4-H). *Anal.* Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 74.03; H, 10.52.

3α,4β,12α-Trihydroxy-5β-cholanoic Acid (1b) Prepared from **8e** (450 mg) by reduction with *tert*-butylamine-borane complex followed by alkaline hydrolysis. Several recrystallizations of the crude product from aqueous MeOH gave the desired **1b** (275 mg, 80%) as colorless crystals, mp 151—152 °C. IR ν_{max} cm^{-1}: 1706 (C=O), 3402, 1036 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.67 (3H, s, 18-Me), 0.93 (3H, s, 19-Me), 0.97 (3H, d, J=6.3 Hz, 21-Me), 3.30 (1H, br m, 3-H), 3.75 (1H, br m, 4-H), 3.92 (1H, m, 12-H). *Anal.* Calcd for $C_{24}H_{40}O_5$: C, 70.55; H, 9.87. Found: C, 70.32; H, 9.63.

The corresponding methyl ester, obtained by the usual method, resisted crystallization, though it was found to be homogeneous in TLC and ^{1}H-NMR analyses. IR ν_{max} cm^{-1}: 1739 (C=O), 3384, 1037 (OH). ^{1}H-NMR δ: 0.68 (3H, s, 18-Me), 0.95 (3H, s, 19-Me), 3.37 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.76 (1H, br m, 4-H), 3.97 (1H, m, 12-H). High-resolution MS: 422.3009 (M$^+$, $C_{25}H_{42}O_5$ requires 422.3032).

3α,4β,7α-Trihydroxy-5β-cholanoic Acid (1c) 10f was hydrolyzed nearly quantitatively by the usual method. Recrystallization of the product from EtOAc gave the desired acid **1c** as colorless needles, mp 131—133 °C. IR ν_{max} cm^{-1}; 1707 (C=O), 3420, 1016, 975 (OH). ^{1}H-NMR ($CDCl_3$+20% DMSO-d_6) δ: 0.65 (3H, s, 18-Me), 0.92 (3H, s, 19-Me), 3.20 (1H, br m, 3-H), 3.81 (1H, m, 7-H), 4.10 (1H, br m, 4-H). *Anal.* Calcd for $C_{24}H_{40}O_5 \cdot 1/2H_2O$: C, 69.02; H, 9.90. Found: C, 69.01; H, 10.10.

The corresponding methyl ester, obtained by the usual method, crystallized from aqueous MeOH as colorless needles, mp 128—130 °C. IR ν_{max} cm^{-1}: 1743 (C=O), 3385, 1017, 975 (OH). ^{1}H-NMR δ: 0.66 (3H, s, 18-Me), 0.92 (3H, d, J=4.5 Hz, 21-Me), 0.94 (3H, s, 19-Me), 3.26 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.88 (1H, m, 7-H), 4.15 (1H, dd, J=10.8, 9.0 Hz, 4-H). *Anal.* Calcd for $C_{25}H_{42}O_5 \cdot 1/2H_2O$: C, 69.57; H, 10.04. Found: C, 69.53; H, 10.12.

3α,4β,7α,12α-Tetrahydroxy-5β-cholanoic Acid (1d) 10g was hydrolyzed nearly quantitatively by the usual method. Recrystallization of the product from aqueous EtOH gave the desired acid **1d** as colorless needles, mp

150—152 °C. IR ν_{max} cm^{-1}: 1703 (C=O), 3396, 1028 (OH). ^{1}H-NMR ($CDCl_3$ + 20% DMSO-d_6) δ: 0.66 (3H, s, 18-Me), 0.91 (3H, s, 19-Me), 0.99 (3H, d, J=6.3 Hz, 21-Me), 3.20 (1H, br m, 3-H), 3.85 (2H, m, 7- and 12-H), 4.13 (1H, br m, 4-H). *Anal.* Calcd for $C_{24}H_{40}O_6 \cdot 3/4H_2O$: C, 65.80; H, 9.55. Found: 66.01; H, 9.47.

The corresponding methyl ester, obtained by the usual method, crystallized from acetone–hexane as colorless prisms, partially melting at 110 °C, and melting at 146—148 °C. IR ν_{max} cm^{-1}: 1740 (C=O), 3410, 1026 (OH). ^{1}H-NMR δ: 0.67 (3H, s, 18-Me), 0.94 (3H, s, 19-Me), 0.97 (3H, d, J= 5.4 Hz, 21-Me), 3.30 (1H, br m, 3-H), 3.66 (3H, s, COOMe), 3.94 (2H, m, 7- and 12-H), 4.23 (1H, br m, 4-H). *Anal.* Calcd for $C_{25}H_{42}O_6$: C, 68.46; H, 9.65. Found: C, 68.30; H, 9.59.

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References and Notes

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